## *Pure species within a continuum of genetic and morphological variation*

Beatty, Gemma E.; Montgomery, W. Ian; Spaans, Florentine; Tosh, David G.; Provan, Jim

*Published in:*
Annals of Botany

*DOI:*
10.1093/aob/mcw002

*Publication date:*
2016

*Citation for published version (APA):*
Beatty, G. E., Montgomery, W. I., Spaans, F., Tosh, D. G., & Provan, J. (2016). Pure species within a continuum of genetic and morphological variation: Sympatric oaks at the edge of their range. *Annals of Botany*, *117*(4), 541-549. https://doi.org/10.1093/aob/mcw002

ORIGINAL ARTICLE

# Pure species in a continuum of genetic and morphological variation: sympatric oaks at the edge of their range

**Gemma E Beatty[1,2,3], W Ian Montgomery[1,2,3], Florentine Spaans[1,2], David G Tosh[1,2] and Jim Provan[1,2,3]**

[1] *School of Biological Sciences, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL*

[2] *Quercus, School of Biological Sciences, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL*

[3] *Institute for Global Food Security, Queen's University Belfast*

Running title: Genetic and morphological variation in oaks

[*]Corresponding author: Dr Jim Provan

E-mail: J.Provan@qub.ac.uk

Tel: +44 (0)28 9097 2489

Fax: +44 (0)28 9097 5588

- *Background and aims* Studies on oaks (*Quercus* spp.) have often been hampered by taxonomic confusion, a situation further compounded by the occurrence of extensive interspecific hybridization. In the present study, we used a combination of genetic and morphological analyses to examine sympatric populations of *Q. petraea* and *Q. robur* at the northwestern edge of their ranges in Northern Ireland, since it had previously been suggested that hybridization could facilitate the apparent rapid, long-distance dispersal of oaks following the glaciations.
- *Methods* Samples were collected from 24 sites across Northern Ireland that had been previously designated as ancient or semi-natural woodland. Genotypes were obtained from a total of 950 trees using twelve nuclear microsatellite loci, and admixture coefficients calculated based on a Bayesian clustering approach. Individuals were also classified as *Q. petraea*, *Q. robur* or hybrids based on two objective morphometric characters shown previously to effectively delineate pure individuals. Genetically "pure" individuals of both species, as defined by the Bayesian clustering, were also genotyped for five chloroplast microsatellites.
- *Key results* Genetic and morphological analyses both indicated the presence of pure individuals of both species, as well as a continuum of intermediates. There was a good agreement between the molecular and morphological classification, with a generally clear separation between pure individuals.
- *Main conclusions* Despite millennia of hybridization and introgression, genetically and morphologically pure individuals of both *Q. petraea* and *Q. robur* can be found at the edge of their range, where both species occur sympatrically. The high proportion of individuals exhibiting introgression compared with previous studies may reflect the historical role of hybridization in facilitating dispersal following the

glaciations. This is further supported by the significantly higher chloroplast diversity in *Q. robur* compared to *Q. petraea*.

**Key words:** Hybridization, introgression, microsatellites, morphological analysis, oak, *Quercus petraea*, *Quercus robur*, species delineation.

# INTRODUCTION

The taxonomy of oaks (*Quercus* spp.) has intrigued and perplexed many eminent scientists since Linnaeus described twelve species in 1753 (Linnaeus 1753). Darwin (1859) highlighted the difficulty in delimiting oak species across Europe in his *On the Origin of Species*, while more regional taxonomic difficulties within the British Isles were addressed by C. C. Babington (1862). These taxonomic struggles have resulted in the number of recognised oak species varying over time, culminating in Schwarz's (1964) *Flora Europaea* entry, which distinguished 320 separate species. The challenge of separating various species complexes into taxonomic units across their geographical ranges remains an area of intensive research and debate, to the extent of proposals conflicting with the conventional Biological Species Concept *sensu* Mayr (1942; van Valen 1976; Kremer & Petit 1993; Manos *et al.* 1999).

One consequence of the sympatric occurrence of many closely related *Quercus* species, and one of the causes of taxonomic confusion is extensive hybridization (Whittemore & Schaal 1991; Rushton 1993). Within the European white oak (Section *Quercus*) complex, *Q. petraea* (Matt.) Liebl. (Sessile oak) and *Q. robur* L. (Pedunculate oak) are the two most widespread and economically important species. They occur sympatrically across temperate Europe, with the range of *Q. petraea* being largely coincident with that of *Q. robur* as far as the eastern borders of Poland and Romania, beyond which *Q. robur* extends to the Urals. Hybrids between the two have been reported throughout their distribution (reviewed in Gardiner 1970; Ortiz-Barrientos & Baack 2014), despite the demonstration of both pre- and postzygotic reproductive barriers (Steinhoff 1993; Bacilieri *et al.* 1996; Streiff *et al.* 1999; Abadie *et al.* 2011). *Q. robur* is an early-successional tree that is associated with base rich, clay soils, often

poorly drained sites and tolerant of waterlogging, whilst *Q. petraea* prefers upland peaty soils and grows in mature forests. Under a model of density-dependent hybridization ("Hubbs' Principle"; Hubbs 1955), patterns of hybridization between *Q. petraea* and *Q. robur* might be expected to reflect the species' ecological preferences (Lagache *et al.* 2013).

*Quercus petraea, Q. robur* and their hybrid *Q.* x *rosacea* (Bechst.) are the only forms within the European white oak complex that occur in Ireland, which represents the extreme northwestern limit of the species' ranges. This species complex recolonized Ireland around 9500 BP from refugia located on the Iberian peninsula (Dumolin-Lapègue *et al.* 1997; Petit *et al.* 2002; Mitchell 2003; Kelleher *et al.* 2004a; Muir *et al.* 2004; Lowe *et al.* 2005). Discriminating between the two species by pollen morphology is not possible, and, thus, it is not possible to determine which species arrived in Ireland first and the lag time, if any, until the subsequent species' arrival. However, due to the recognised status of *Q. robur* as a colonising species, it may be postulated that this species was the first to arrive. Oak woodlands subsequently dominated Ireland until the Neolithic, when use of the trees for building instigated a steep decline, and by the 1600s only 2-3% of Ireland's forest cover remained (Mitchell 1995; Rackham 1995), making Ireland the least wooded region in Europe with the exception of Iceland.

The occurrence of oak at high latitudes soon after the retreat of the ice formed the basis of a long-standing debate in biogeography ("Reid's Paradox"), since the apparent speed of recolonization was believed to vastly exceed the species' dispersal capacities (Reid 1899; Provan & Bennett 2008). It has been suggested, however, that hybridization between *Q. petraea* and *Q. robur* could facilitate rapid colonization through a process of differential dispersal and asymmetric pollen-mediated

introgression (Petit *et al.* 2003). Ireland, being located on the northwesten edge of the species' distribution, would have to have been recolonized particularly quickly due to the increase in sea levels following the glaciations, the aim of the present study was to determine the genetic composition of oaks in Northern Ireland. Here, we investigate genetic and morpohological variation in Irish oak to examine how *Q. petraea* and *Q. robur* maintain genetic and phenotypic integrity whilst indulging in promiscuity during alternative periods of extreme environmental change and stasis. We sampled "blind" (*sensu* Lepais *et al.* 2009), rather than *a priori* identifying individuals as either *Q. petraea* or *Q. robur*, in order to give a true representation of both species and the entire spectrum of genetic intermediates resulting from nearly 10,000 years of hybridization and backcrossing. We also analysed levels and patterns of nuclear and chloroplast genetic diversity in genetically "pure" individuals of both species to determine if there was any bias in levels of chloroplast diversity. Under the scenario outlined by Petit *et al.* (2003), we would expect to see similar levels of nuclear diversity, but far higher levels of chloroplast diversity in *Q. robur*, since acorns from this species would be responsible for the majority of colonization events.

## MATERIALS AND METHODS

### *Sampling and DNA extraction*

Samples were collected from 24 sites across Northern Ireland that had been previously designated as ancient or semi-natural woodland based on data collected for the Woodland Trust Inventory of ancient and long-established woodland in Northern Ireland (www.backonthemap.org.uk; Fig. 1 and Table 1). For genetic analyses, a single leaf was collected from up to 48 trees per site and stored in silica gel, and GPS coordinates recorded for every tree. DNA was extracted using the CTAB method of Doyle and Doyle (1987). Nuclear genotypes were obtained for between 17 and 47 individuals per population (Table 1; total = 950; mean = 39.583). For morphometric analyses, three leaves were taken from the same 48 trees per population, with the exception of Banagher Glen (23) and Correl Glen (47; total = 1126; mean 46.875). Fully expanded, open canopy leaves were collected to minimize any effects of environmental factors such as exposure / shade.

### *Genotyping*

All trees were genotyped for twelve nuclear microsatellite loci: MsQ13, QpZAG15, QpZAG110, QrZAG7, QrZAG20, QrZAG96, QrZAG112, PIE020, PIE102, PIE223, PIE239 and PIE271 (Guichoux *et al.* 2011). Forward primers included a 19 bp M13 tail (CACGACGTTGTAAAACGAC) and reverse primers included a 7 bp tail (GTGTCTT). PCR was carried out in a total volume of 10 μl containing 100 ng genomic DNA, 5 pmol of 6-FAM-, HEX- or PET-labelled M13 primer, 0.05 pmol of each M13-tailed forward primer, 5 pmol each reverse primer, 1x PCR reaction buffer, 200 μM each dNTP, 2.5 mM $MgCl_2$ and 0.25 U GoTaq Flexi DNA polymerase

(Promega, Sunnyvale, CA, USA). PCR was carried out on a MWG Primus thermal cycler (Ebersberg, Germany) using the following conditions: initial denaturation at 94 $^{o}$C for 3 min followed by 40 cycles of denaturation at 94 $^{o}$C for 30 s, annealing at 56 $^{o}$C for 30 s, extension at 72 $^{o}$C for 30 s and a final extension at 72 $^{o}$C for 5 min. Genotyping was carried out on an AB3730xl capillary genotyping system (Applied Biosystems, Foster City, CA, USA). Allele sizes were scored using the GENEMAPPER software package (v4.1; Applied Biosystems) using LIZ-500 size standards, and were checked by comparison with previously sized control samples. Chromatograms were all inspected visually.

All trees were also genotyped for five chloroplast microsatellite loci: μdt1, μdt3, μdt4, μcd5 and μkk4 (Deguilloux *et al.* 2003). PCR and genotyping were carried out as described above, except that an annealing temperature of 44 $^{o}$C was used over 30 cycles.

*Genetic data analysis*

GENEPOP (V3.4; Raymond and Rousset, 1995) was used to test for linkage disequilibrium between nuclear microsatellite loci. To assess the levels of admixture within individuals, Bayesian model-based clustering based on nuclear microsatellites was carried out using STRUCTURE (V 2.2; Pritchard *et al.* 2000). The number of clusters was set to $K = 2$ to represent the two putative parental species, *Quercus petraea* and *Q. robur*. The program was run using 50,000 burn-in iterations followed by 500,000 Markov Chain Monte Carlo iterations. The analysis was carried out ten times and mean values of the admixture coefficient, $Q$, were calculated for each individual.

Levels and patterns of genetic diversity were calculated for populations containing at least ten "pure" individuals of either species, based on the $Q$ values ($Q \leq 0.1$ for *Q. petraea* and $Q \geq 0.9$ for *Q. robur*; Table 2). Levels of observed ($H_O$) and expected ($H_E$)

heterozygosity and levels of allelic richness ($A_R$) were calculated using the FSTAT software package (V2.9.3.2; Goudet 2001). Chloroplast microsatellite allele sizes were combined into haplotypes, and levels of genetic diversity ($H$) based on haplotype frequencies were calculated using the ARLEQUIN software package (V3.5.1.2; Excoffier & Lischer 2010). To account for differences in sample sizes, levels of haplotype richness ($R_h$) were also calculated using HAPLOTYPE ANALYSIS (V1.05; Eliades and Eliades 2009).

*Morphometric analysis*

Morphometric analysis employed two objective characters shown previously to effectively delineate pure individuals of *Q. petraea* and *Q. robur*, namely the presence (*Q. petraea*) or absence (*Q. robur*) of stellate hairs (Aas 1995), and the ratio of petiole length to lamina length (Cousens 1963; Kelleher *et al.* 2004b; *Q. petraea* $\geq 0.1$, *Q. robur* $< 0.1$). Individuals exhibiting both species-specific characters (petiole/lamina ratio averaged across the three leaves examined) were assigned to that species, with the remainder being classed as hybrids. Although our approach is not directly comparable with most previous morphological studies, which use multiple characters and multivariate analysis to classify individuals, it is categorical, which makes delineation simpler.

# RESULTS

*Genetic analysis*

No significant evidence of consistent linkage disequilibrium (i.e. involving the same loci) was detected between any of the twelve nuclear microsatellites analysed (58 out of 1586 tests). The STRUCTURE analysis indicated a range of admixture ($Q$) values. Comparison with the morphometric analysis indicated that $Q = 0.00$ corresponded to *Q. petraea* and $Q = 1.00$ to *Q. robur*. The overall spectrum of $Q$ values is shown in Figure 2. Spectra for individual populations are shown in Figure 3.

For genetically "pure" individuals, levels of allelic richness ($A_R$) ranged from 4.788 (Castle Archdale) to 5.600 (Roe Valley; mean = 5.152) in *Q. petraea*, and from 4.556 (Gosford Park) to 5.163 (Fardross Forest; mean = 4.807) in *Q. robur* (Table 2). Levels of observed ($H_O$) and expected ($H_E$) heterozygosity ranged from 0.619 (Castle Archdale) to 0.714 (Hollymount; mean = 0.653) and from 0.677 (Errigal Glen) to 0.766 (Roe Valley; mean = 0.714) respectively in *Q. petraea*, and from 0.575 (Gosford Park) to 0.781 (Belle Isle; mean = 0.663) and from 0.676 (Gortin Glen) to 0.760 (Belle Isle; mean = 0.720) respectively for *Q. robur* (Table 2).

A total of 14 chloroplast haplotypes were detected, six of which were found in a single individual (Table 2). Only the two most common haplotypes (H1 and H2) were found in *Q. petraea*, and nine of the ten populations were fixed for a single haplotype, the exception being Glenarm, which exhibited a haplotype diversity ($H$) of 0.282 and a haplotype richness ($R_h$) of 0.923. *Q. robur* populations exhibited far higher chloroplast genetic diversity, with levels of $H$ and $R_h$ ranging from 0.167 (Barnett's Demesne) to 0.723 (Gortin Glen; mean = 0.464), and from 0.750 (Barnett's Demesne) to 2.743 (Fardross Forest; mean = 1.955) respectively. Levels of $A_R$ were significantly lower in

*Q. robur* than in *Q. petraea* (Wilcoxon Test: W = 67; $P = 0.01554$), whilst there was no significant difference in $H_E$ (Wilcoxon Test: W = 33; $P = 0.56340$; Figure 4). Levels of both $H$ and $R_h$ were significantly lower in *Q. petraea* than in *Q. robur* (Wilcoxon Test: W = 3; $P = 0.00053$ and W = 2; $P = 0.00037$ respectively).

*Morphometric analysis*

Morphometric analysis based on two discriminatory characters classified 345 individuals (30.67% of individuals studied) as *Q. petraea* and 251 individuals (22.31%) as *Q. robur*. The remaining 529 (47.02%) were classified as hybrids. The geographical distribution of the three classes by population is shown in Figure 5. Based on morphology, only two of the 24 populations studied (Ness Wood and Correl Glen) had pure individuals of only one species (*Q. petraea*) along with putative hybrid individuals. *Q* values for trees morphometrically assigned to *Q. petraea* (mean = 0.212; median = 0.125) were significantly different from those for trees assigned to *Q. robur* (mean = 0.821; median = 0.906; Figure 6). Boxplots showing distributions of *Q* values by population are given in Figure S1, Supporting Information. In 15 out of 18 populations where more than one individual of each species was analyzed, *Q* values for *Q. petraea* and *Q. robur* were significantly different (Mann-Whitney U test).

# DISCUSSION

The results of the present study indicate that oak woodlands in Northern Ireland, which represents the northwestern edge of their distribution range, comprise both a morphological and genetic continuum of individuals that range from pure *Q. petraea* to pure *Q. robur*, with varying degrees of hybridization and introgression in intermediate individuals. Using the genetic criteria for "pure" individuals as defined by Lepais *et al.* (2009; admixture coefficient $Q < 0.1$ for *Q. petraea* or $> 0.9$ for *Q. robur*), *ca.* 23% of trees were classified as pure *Q. petraea*, whilst *ca*. 22% were classified as pure *Q. robur*. There was good agreement between the molecular and morphological classification, with a generally clear separation between pure individuals. This was particularly apparent in the two populations from which no individuals were identified morphologically as *Q. robur*, Ness Wood and Correl Glen, with no *Q* scores of greater than 0.6 in the former or greater than 0.7 in the latter. Previous morphological studies in Ireland had suggested that the two form distinct species, but also that some hybrids occur (Carlisle & Brown 1965; Minihan & Rushton 1984; Rushton 1983, 1993; Kelleher *et al.* 2004b). In their *Flora of County Fermanagh*, Forbes and Northridge (2012) stated that due to difficulty in species identification as a result of introgression, they had amalgamated their accounts of both oak species under *Q. petraea*. Their reasoning was that *Q. petraea* was "... generally regarded as a good, homogenous species ...", but a comparison of our morphological and genetic data indicate that both species are represented by genetically and morphologically distinct individuals. A previous study on 24 populations across Ireland using AFLPs found little separation of the two species, despite morphological distinctiveness (Kelleher *et al.* 2005). This contrast to the findings of the present study could be due to the use of dominant markers

and the low number of samples (five) analyzed from each population, although an earlier AFLP study on Flemish populations using larger sample sizes (mean = 18.8 per population) showed clear molecular and morphological discrimination (Coart *et al.* 2002).

The use in the present study of twelve microsatellite markers that share many alleles between the two species means that it is not possible to assign individuals to various hybrid and backcross classes with any degree of certainty (Vaha & Primmer 2006). Nevertheless, based on the distribution of admixture coefficients, it would seem that relatively few first generation ($F_1$) hybrids are represented in the samples, and that backcrossing to either putative parental species predominates in individuals exhibiting evidence of introgression. A similar scenario was described previously by Lepais *et al.* (2009), and thought to originate through a combination of pre-reproductive barriers such as density-dependent pollination and intrinsic pollen discrimination favouring intraspecific crosses and backcrosses over interspecific mating (Lepais & Gerber 2011). Both the genetic and the morphological data indicate that the majority of the woodlands examined in the present study tend to be comprised of one or other of the two parental species, plus hybrids. Again, this is most likely a result of relative abundance of parental species and pollen discrimination (Lepais *et al.* 2009; Lepais & Gerber 2011; Legache *et al.* 2014), but it has also been shown that other environmental factors including fine-scale spatial organization and selection can control hybridization (Gugerli *et al.* 2007; Lagache *et al.* 2013). This complex interplay of factors may explain not only the regular occurrence of single species / hybrid dominated woodlands, but also the occasional sites where all three classes are found, such as Hillsborough.

Although 45% of the individuals analyzed genetically were assigned to pure *Q. petraea* or *Q. robur*, the remaining 55% exhibiting evidence of introgression is far

higher than the levels reported in a previous "blind" study by Lepais *et al.* (2009), which also analyzed two other white oak species, *Q. pubescens* and *Q. pyrenaica*. In it, they assigned 48.9% and 32.1% of individuals to *Q. robur* and *Q. petraea* respectively from an exhaustively sampled stand containing only the two species, with 5.7% of individuals classed as hybrids between the two, the remaining 14.7% exhibiting introgression from either *Q. pubescens* or *Q. pyrenaica* as a result of pollen immigration. The high values observed in the present study could reflect the historical importance of hybridization in facilitating the long-distance recolonization of Ireland from Iberia after the last glacial period, which ended approximately 11.5 kya. Although most palynological analyses do not discriminate between the two species, the pioneer nature of *Q. robur*, with its acorns more likely to be dispersed over long distances than those of *Q. petraea* (Jones 1959; Petit *et al.* 2003), suggests that it colonized first. Subsequent pollen flow from *Q. petraea*, followed by hybridization, would facilitate the dispersal of the later successional species into the area colonized by the pioneer, and rapid unidirectional backcrossing could restore both species over time (Petit *et al.* 2003). The levels and patterns of genetic diversity observed at nuclear and chloroplast loci in genetically "pure" individuals are completely consistent with this scenario. Previous studies have demonstrated that chloroplast haplotypes are shared between the two species (Dumolin-Lapègue *et al.* 1997; Petit *et al.* 2002; Cottrell *et al.* 2002) but, despite this, chloroplast genetic diversity is significantly higher in *Q. robur*, suggesting a great predominance of *Q. robur* acorns during the recolonization process. Generations of assortative mating, as a result of environmental and intrinsic barriers would facilitate adaptation to prevailing climatic and edaphic conditions, and ultimately lead to the present day observed distributions of the species and their hybrids at the limit of their distribution ranges.

ACKNOWLEDGEMENTS

This study was commissioned and funded by the Natural Heritage Research Partnership between the Northern Ireland Environment Agency (NIEA), Department of the Environment Northern Ireland, and *Quercus*, Queen's University Belfast. We are grateful to all the landowners who gave us permission to sample on their land, to all who helped with sampling, to Remy Petit for helpful discussions, and to John Farren, who acted as NIEA Client Officer.

# LITERATURE CITED


**Aas G. 1995.** Die Behaarung der Blätter von Traubeneiche und Stieleiche (*Quercus petraea* und *Quercus robur*): Variabilität und taxonomische Bedeutung. *Mitteilungen aus der Forstlichen Versuchsanstalt Rheinland-Pflaz* **34**: 297–309.

**Abadie P, Roussell G, Dencausse B *et al*. 2011.** Strength, diversity and plasticity of postmating reproductive barriers between two hybridizing oak species (*Quercus robur* L. and *Quercus petraea* (Matt) Liebl.). *Journal of Evolutionary Biology* **25**:157-173.

**Babington CC. 1862.** *Manual of British Botany*. 5th edition. John van Voorst, London, UK.

**Bacilieri R, Ducousso A, Petit RJ, Kremer A. 1996.** Mating system and asymmetric hybridization in a mixed stand of European oaks. *Evolution* **50**:900-908.

**Carlisle A, Brown AHF. 1965.** The assessment of the taxonomic status of mixed oak (*Quercus* spp.) populations. *Watsonia* **6**:120-127.

**Coart E, Lamote V, de Loose M, van Bockstaele E, Lootens P, Roldán-Ruiz I. 2002.** AFLP markers demonstrate local genetic differentiation between two indigenous oak species (*Quercus robur* L. and *Quercus petraea* (Matt) Liebl.) in Flemish populations. *Theoretical and Applied Genetics* **105**:431-439.

**Cottrell JE, Munro RC, Tabbener HE, Gillies ACM, Forrest GI, Deans JD, Lowe AJ. 2002.** Distribution of chloroplast DNA variation in British oaks (*Quercus robur* and *Quercus petraea*): the influence of postglacial colonisation and human management. *Forest Ecology and Management* **156**:181-195.

**Cousens JE. 1963.** Variation of some diagnostic characters of the sessile and pedunculate oaks and their hybrids in Scotland. *Watsonia* **5**:273-286.

**Darwin CR. 1853.** *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life.* John Murray, London, UK.

**Doyle JJ, Doyle JL. 1987.** A rapid DNA isolation procedure for small quantities of fresh leaf material. *Phytochemical Bulletin* **19**:11-15.

**Dumolin-Lapègue S, Demesure B, Finseche S, Le Corre V, Petit RJ. 1997.** Phylogoegraphic structure of white oaks throughout the European continent. *Genetics* **146**:1475-1487.

**Eliades N-G, Eliades DG. 2009.** HAPLOTYPE ANALYSIS: software for analysis of haplotype data. www.uni-goettingen.de/en/134935.html.

**Excoffier L, Lischer HEL. 2010.** Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**:564-567.

**Forbes RS, Northridge RH. 2012.** *The Flora of County Fermanagh*. National Museums Northern Ireland.

**Gardiner AS. 1970.** Pedunculate and Sessile oak (*Quercus robur* L. and *Quercus petraea* (Mattuschka) Liebl.): a review of the hybrid controversy. *Forestry* **43**:151-160.

**Goudet J. 2001.** FSTAT, version 2.9.3, A program to estimate and test gene diversities and fixation indices. http://www2.unil.ch/popgen/softwares/fstat.htm.

**Gugerli F, Walser J-C, Dounavi K, Holderegger R, Finkeldey R. 2007.** Coincidence of small-scale spatial discontinuities in leaf morphology and nuclear microsatellite variation of *Quercus petraea* and *Q. robur* in a mixed forest. *Annals of Botany* **99**:713-722

**Guichoux E, Lagache L, Wagner S, Léger P, Petit RJ. 2011.** Two highly validated multiplexes (12-plex and 8-plex) for species delimitation and parentage analysis in oaks (*Quercus* spp.). *Molecular Ecology Resources* **11**:578-585.

**Hubbs CL. 1955.** Hybridization between fish in nature. *Systematic Zoology* **4**:1-20.

**Jones EW. 1959.** Biological flora of the British Isles. *Quercus* L. *Journal of Ecology* **47**:169-222.

**Kelleher CT, Hodkinson TR, Kelly DL, Douglas GC. 2004a.** Characterisation of chloroplast DNA haplotypes to reveal the provenance and genetic structure of oaks in Ireland. *Forest Ecology and Management* **189**:123-131.

**Kelleher CT, Kelly DL, Hodkinson TR. 2004b.** Species status, hybridisation and geographic distribution of Irish populations of *Quercus petraea* (Matt.) Liebl. and *Q. robur* L. *Watsonia* **25**:83-97.

**Kelleher CT, Hodkinson TR, Douglas GD, Kelly DL. 2005.** Species distinction in Irish populations of *Quercus petraea* and *Q. robur*: morphological versus molecular analyses. *Annals of Botany* **96**:1237-1246.

**Kelly DL. 1996.** A short guide to Irish oaks. *Moorea* 11: 24-28.

**Kremer A, Petit RJ. 1993.** Gene diversity in natural populations of oak species. *Annales des Sciences Forestières* **50**:186s-202s.

**Lagache L, Klein EK, Guichoux E, Petit RJ. 2013.** Fine-scale environmental control of hybridization in oaks. *Molecular Ecology* **22**:423-436.

**Lagache L, Klein EK, Ducousso A, Petit RJ. 2014.** Distinct male reproductive strategies in two closely related oak species. *Molecular Ecology* **23**:4331-4343.

**Lepais O, Petit RJ, Guichoux E *et al.* 2009.** Species relative abundance and direction of introgression in oaks. *Molecular Ecology* **18**:2228-2242.

**Lepais O, Gerber S. 2011.** Reproductive patterns shape introgression dynamics and species succession within the European white oak species complex. *Evolution* **65**:156-170.

**Linnaeus C. 1753.** *Species Plantarum 2*, Stockholm, Sweden.

**Lowe A, Unsworth C, Gerber S *et al.* 2005.** Route, speed and mode of oak postglacial colonisation across the British Isles: integrating molecular ecology, palaeoecology and modelling approaches. *Botanical Journal of Scotland* **57**:59-91.

**Manos PS, Doyle JJ, Nixon KC. 1999.** Phylogeny, biogeography and processes of molecular differentiation in *Quercus* Subgenus *Quercus* (Fagaceae). *Molecular Phylogenetics and Evolution* **12**:333-349.

**Mayr E. 1942.** *Systematics and the Origin of Species from the Viewpoint of a Zoologist.* Columbia University Press, New York, USA.

**Minihan VB, Rushton RS. 1984.** The taxonomic status of oaks (*Quercus* spp.) in Breen Wood, Co. Antrim, Northern Ireland. *Watsonia* **15**:27-32.

**Mitchell FJG. 1995.** The dynamics of irish post-glacial forests. In: Mac an tSoir SS (ed) *Wood, trees and forests in Ireland.* pp. 13-22, Royal Irish Academy, Dublin, Ireland.

**Mitchell FJG. 2003.** Natural invaders: the postglacial tree colonization of Ireland. In: Murray DA (ed) *Biological invaders: the impact of exotic species.* pp. 2-13, Royal Irish Academy, Dublin, Ireland.

**Muir G, Lowe AJ, Fleming CC, Vogl C. 2004.** High nuclear genetic diversity, high levels of outcrossing, and low differentiation among remnant populations of *Quercus petraea* at the margin of its range in Ireland. *Annals of Botany* **93**:691-697.

**Ortiz-Barrientos D, Baack EJ. 2014.** Species integrity in trees. *Molecular Ecology* **23**:4188-4191.

**Petit RJ, Csaikl UM, Bordács S *et al.* 2002.** Chloroplast DNA variation in European white oaks: Phylogeography and patterns of diversity based on data from over 2600 populations. *Forest Ecology and Management* **156**:5-26.

**Petit RJ, Bodénès C, Ducousso A, Roussel G, Kremer A. 2003.** Hybridization as a mechanism of invasion in oaks. *New Phytologist* **161**:151-164.

**Pritchard JK, Stephens M, Donnelly P. 2000.** Inference of population structure using multilocus genotype data. *Genetics Society of America* **155**:945-959.

**Provan J, Bennett KD. 2008.** Phylogeographic insights into cryptic glacial refugia. *Trends in Ecology and Evolution* **23**:564-571.

**Rackham O. 1995.** Looking for ancient woodland in Ireland. In: Mac an tSoir SS (ed) *Wood, Trees and Forests in Ireland.* pp. 1-12, Royal Irish Academy, Dublin, Ireland.

**Raymond M, Rousset F. 1995.** GENEPOP (version 1.2): population genetic software for exact tests and ecumenicism. *Journal of Heredity* **86**:248-249.

**Reid C. 1899.** *The Origin of the British Flora*. Dulau, London, UK.

**Rushton BS. 1983.** An analysis of variation of leaf characters in *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. population samples from Northern Ireland. *Irish Forestry* **40**:52-77.

**Rushton BS. 1993.** Natural hybridization within the genus *Quercus* L. *Annales des Sciences Forestières* 50: 73s-90s.

**Schwarz O. 1964.** *Quercus* L. In: Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SN, Webb DA (eds) *Flora Europaea* Vol. 1: *Lycopodiaceae to Plantanaceae*. pp 61-64. Cambridge University Press, Cambridge, UK.

**Steinhoff S. 1993.** Results of species hybridization with *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. *Annals of Forest Science* **50**:137s-143s.

**Streiff R, Ducousso A, Lexer C, Steinkellner H, Gloessl J, Kremer A. 199.** Pollen dispersal inferred from paternity analysis in a mixed oak stand of *Quercus robur* L. and *Q. petraea* (Matt.) Liebl. *Molecular Ecology* **8**:831-841.

**Vaha JP, Primmer CR. 2006.** Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. *Molecular Ecology***15**:63-72.

**van Valen L. 1976.** Ecological species, multi-species and oaks. *Taxon* **25**:233-239.

**Weir BS, Cockerham CC. 1984.** Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358-1370.

**Whittemore AT, Schaal BA. 1991.** Interspecific gene flow in sympatric oaks. *Proceedings of the National Academy of Sciences USA* **88**:2540-2544.

TABLE 1 Details of populations studied. *N* – number of individuals analysed.

| *No* | *Name* | *Lat (N)* | *Long (W)* | *N* |
|---|---|---|---|---|
| 1 | Portaferry | 54.391 | 5.565 | 34 |
| 2 | Hollymount | 54.322 | 5.751 | 41 |
| 3 | Glenarm | 54.964 | 5.955 | 37 |
| 4 | Barnett's Demesne | 54.552 | 5.960 | 46 |
| 5 | Hillsborough | 54.459 | 6.083 | 41 |
| 6 | Rostrevor | 54.093 | 6.190 | 44 |
| 7 | Rea's Wood | 54.705 | 6.229 | 47 |
| 8 | Breen Wood | 55.138 | 6.238 | 32 |
| 9 | Portglenone | 54.863 | 6.472 | 41 |
| 10 | Gosford Park | 54.304 | 6.523 | 34 |
| 11 | Peatlands Park | 54.483 | 6.612 | 35 |
| 12 | Errigal Glen | 54.971 | 6.733 | 40 |
| 13 | Drum Manor | 54.639 | 6.815 | 43 |
| 14 | Roe Valley | 55.025 | 6.939 | 38 |
| 15 | Banagher Glen | 54.884 | 6.954 | 17 |
| 16 | Ness Wood | 54.947 | 7.181 | 42 |
| 17 | Gortin Glen | 54.667 | 7.233 | 41 |
| 18 | Fardross Forest | 54.374 | 7.268 | 45 |
| 19 | Crom | 54.170 | 7.451 | 43 |
| 20 | Belle Isle | 54.245 | 7.564 | 46 |
| 21 | Sloughan Glen | 54.622 | 7.564 | 34 |
| 22 | Castle Archdale | 54.484 | 7.722 | 46 |
| 23 | Marble Arch | 54.264 | 7.812 | 43 |
| 24 | Correl Glen | 54.439 | 7.885 | 40 |

TABLE 2 Diversity statistics by population for "pure" individuals (see text for details). $N$ – number of individuals analysed; $A_R$ – allelic richness; $H_O$ – observed heterozygosity; $H_E$ – expected heterozygosity; H1-H8 – frequency of chloroplast haplotypes; Un – unique haplotype; $H$ – gene diversity; $R_h$ – haplotype richness.

| *Species* | *No* | *Name* | *Nuclear* | | | | *Chloroplast* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | N | $A_R$ | $H_O$ | $H_E$ | N | *H1* | *H2* | *H3* | *H4* | *H5* | *H6* | *H7* | *H8* | *Un* | H | $R_h$ |
| *Q. petraea* | 2 | Hollymount | 14 | 5.256 | 0.714 | 0.693 | 14 | 14 | - | - | - | - | - | - | - | - | - | - |
| | 3 | Glenarm | 13 | 4.914 | 0.642 | 0.711 | 13 | 2 | 11 | - | - | - | - | - | - | - | 0.282 | 0.923 |
| | 6 | Rostrevor | 23 | 5.472 | 0.680 | 0.725 | 23 | 23 | - | - | - | - | - | - | - | - | - | - |
| | 8 | Breen Wood | 17 | 5.045 | 0.641 | 0.731 | 17 | 17 | - | - | - | - | - | - | - | - | - | - |
| | 12 | Errigal Glen | 12 | 4.858 | 0.699 | 0.677 | 12 | 12 | - | - | - | - | - | - | - | - | - | - |
| | 14 | Roe Valley | 10 | 5.600 | 0.697 | 0.766 | 9 | 9 | - | - | - | - | - | - | - | - | - | - |
| | 16 | Ness Wood | 24 | 5.197 | 0.557 | 0.721 | 24 | 24 | - | - | - | - | - | - | - | - | - | - |
| | 21 | Sloughan Glen | 14 | 4.995 | 0.634 | 0.706 | 14 | - | 14 | - | - | - | - | - | - | - | - | - |
| | 22 | Castle Archdale | 20 | 4.788 | 0.619 | 0.692 | 20 | - | 20 | - | - | - | - | - | - | - | - | - |
| | 24 | Correll Glen | 22 | 5.391 | 0.642 | 0.721 | 22 | 22 | - | - | - | - | - | - | - | - | - | - |
| *Q. robur* | 1 | Portaferry | 12 | 5.007 | 0.588 | 0.740 | 12 | 9 | 2 | - | - | - | - | - | - | 1 | 0.204 | 1.705 |
| | 4 | Barnett's Demesne | 12 | 4.703 | 0.729 | 0.732 | 12 | 11 | - | - | - | - | - | - | - | 1 | 0.167 | 0.750 |
| | 9 | Portglenone | 18 | 4.728 | 0.705 | 0.720 | 18 | 16 | 2 | - | - | - | - | - | - | - | 0.209 | 0.765 |
| | 10 | Gosford Park | 16 | 4.556 | 0.575 | 0.679 | 15 | 2 | 10 | 1 | 1 | - | 1 | - | - | - | 0.562 | 2.657 |
| | 13 | Drum Manor | 13 | 4.727 | 0.668 | 0.713 | 11 | - | 6 | - | 4 | - | - | - | - | 1 | 0.618 | 1.818 |
| | 17 | Gortin Glen | 25 | 4.564 | 0.579 | 0.676 | 24 | - | 1 | 16 | - | 3 | - | 1 | 2 | 1 | 0.723 | 2.520 |
| | 18 | Fardross Forest | 31 | 5.163 | 0.680 | 0.741 | 31 | 9 | 13 | 1 | 6 | - | 1 | - | - | 1 | 0.551 | 2.743 |
| | 20 | Belle Isle | 18 | 5.011 | 0.781 | 0.760 | 16 | 5 | 8 | 1 | - | - | - | 1 | - | 1 | 0.683 | 2.683 |

FIGURE LEGENDS

FIG. 1. Locations of sites sampled in this study. Numbers correspond to those in Table 1.

FIG. 2. Distribution spectrum of admixture coefficient (*Q*) values across all samples (*N* = 955).

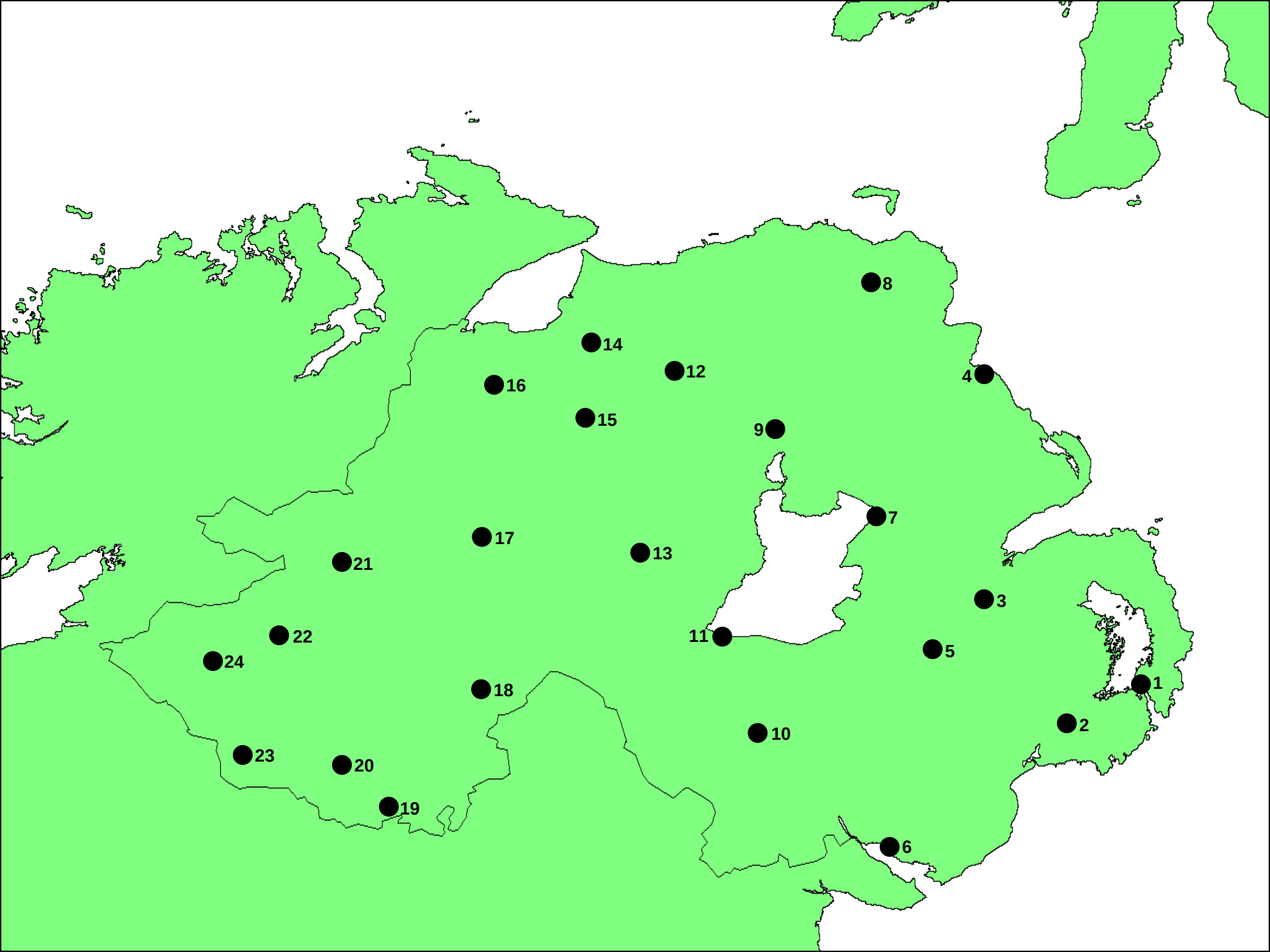
FIG. 3. Distribution spectra of admixture coefficient (*Q*) values by population. *Q* value classes are given on the x-axis, whilst numbers of individuals are given on the y-axis. Note that values on the y-axis vary from site to site.

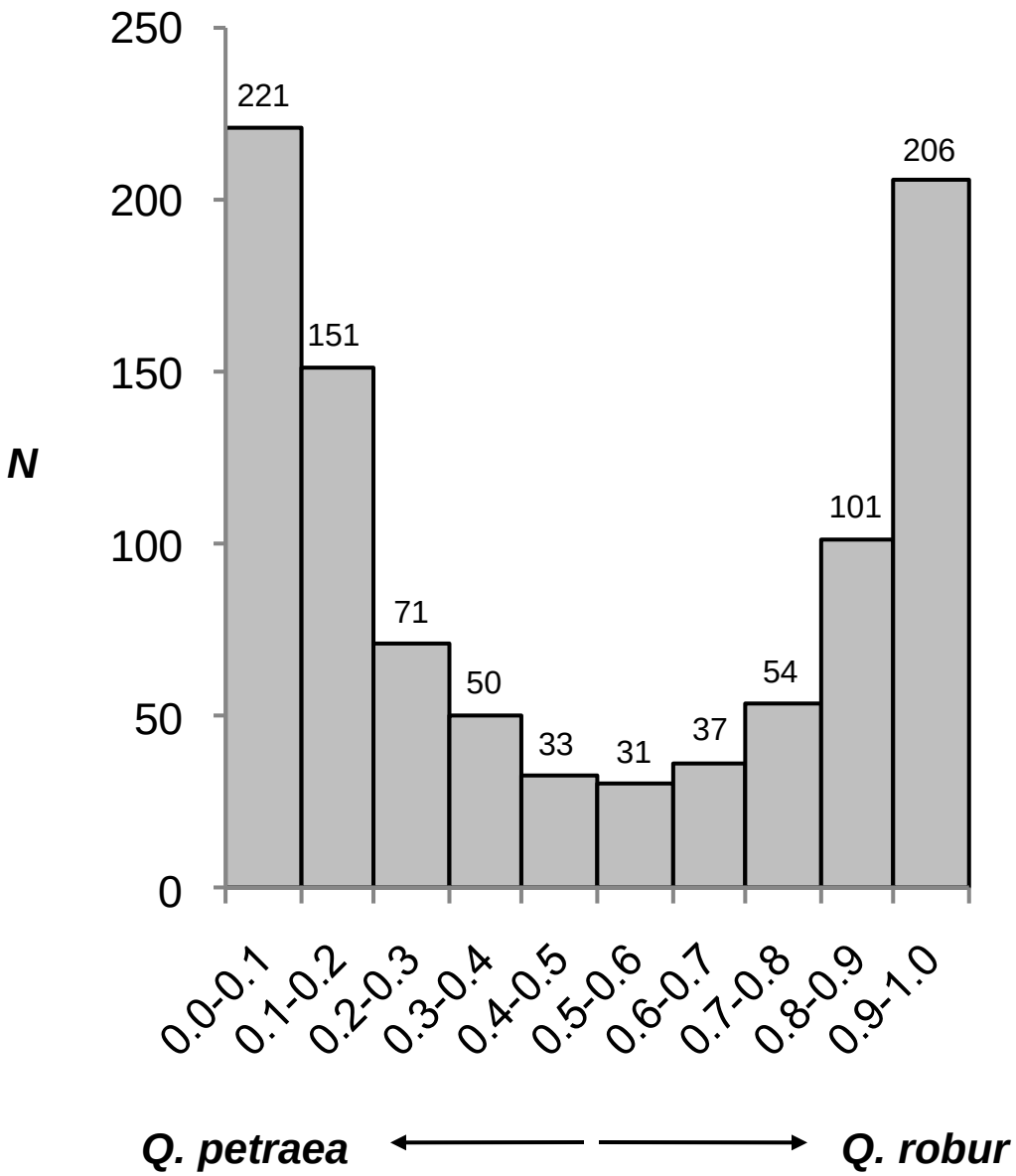
FIG 4. Boxplots showing comparisons of levels of nuclear ($A_R$ and $H_E$) and chloroplast ($H$ and $R_H$) genetic diversity between"pure" *Q. petraea* and *Q. robur*.

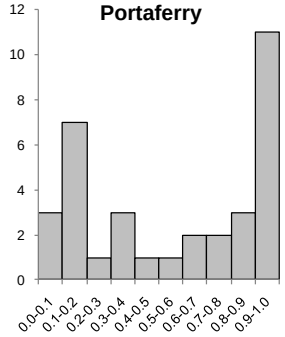
FIG. 5. Geographical distribution of *Quercus petraea* (black), *Q. robur* (white) and hybrids (grey) by population based on morphological analyses.

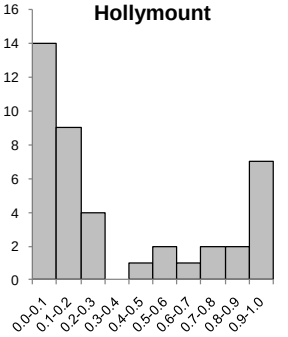
FIG 6. Boxplot showing admixture coefficient (*Q*) values for *Quercus petraea*, *Q. robur* and hybrids. Solid bar represents the median.

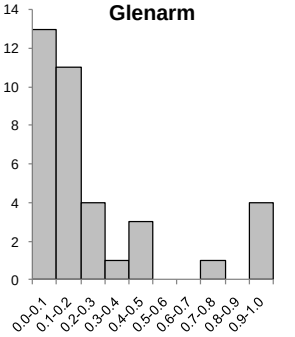
FIG S1. Boxplots showing admixture coefficient (*Q*) values for *Quercus petraea*, *Q. robur* and hybrids by population. Asterisks indicate significant differences (Mann-Whitney test).

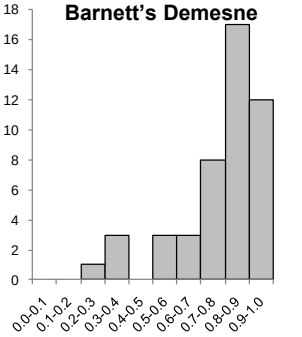

8
14
12
4
16
15
9
7
17
13
21
3
22
11
5
24
18
1
10
2
23
20
19
6

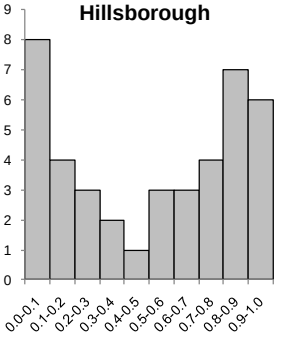

250
200
150
100
50
0
N
221
151
71
50
33
31
37
54
101
206
0.0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0
Q. petraea
Q. robur

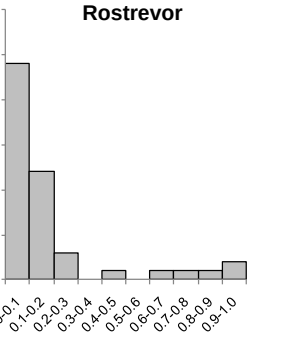

Portaferry
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

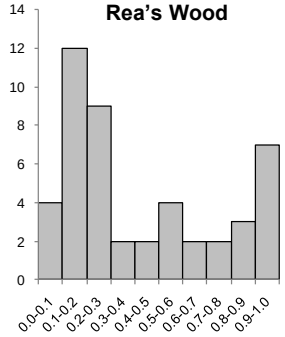

Hollymount
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

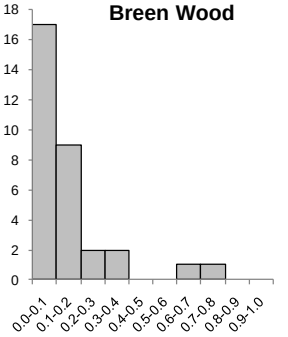

Glenarm
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

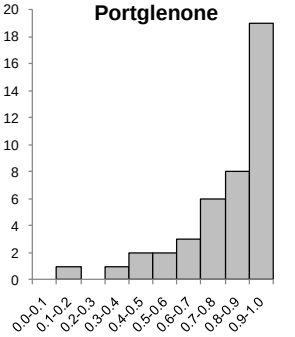

Barnett's Demesne
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

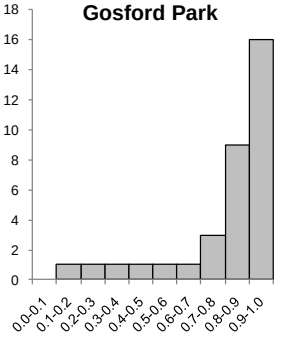

Hillsborough
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

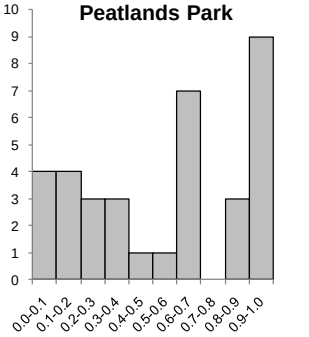

Rostrevor
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

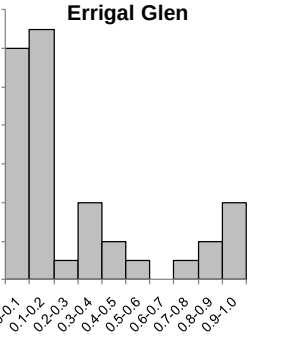

Rea's Wood
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

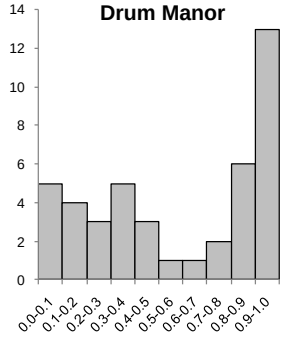

Breen Wood
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

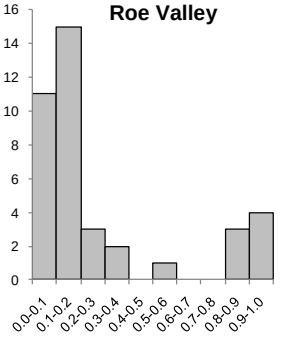

Portglenone
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

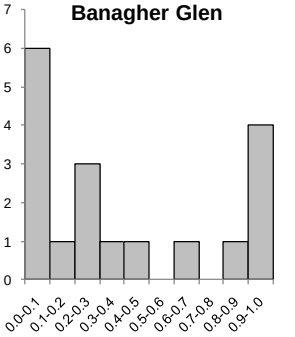

Gosford Park
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

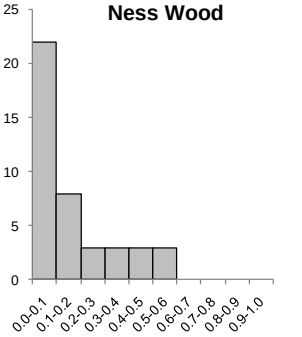

Peatlands Park
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

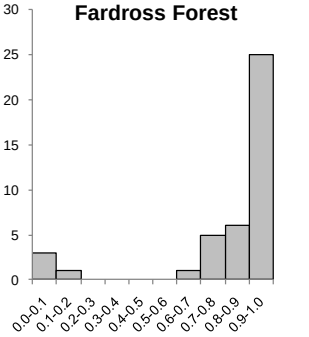

Errigal Glen
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

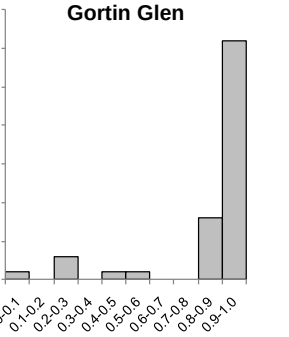

Drum Manor
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

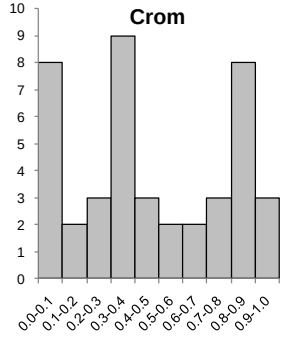

Roe Valley
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

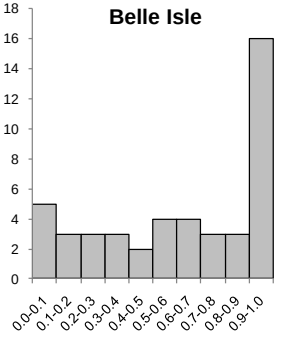

Banagher Glen
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

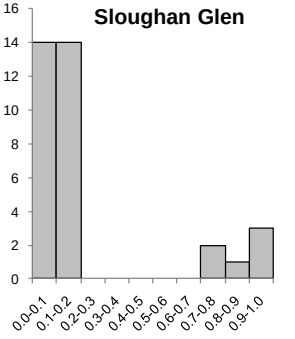

Ness Wood
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

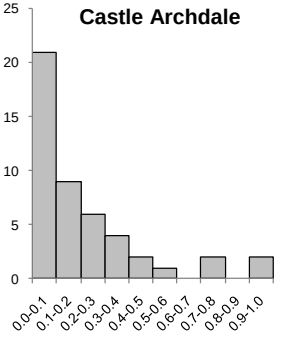

Fardross Forest
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

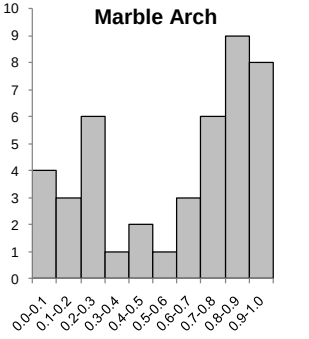

Gortin Glen
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

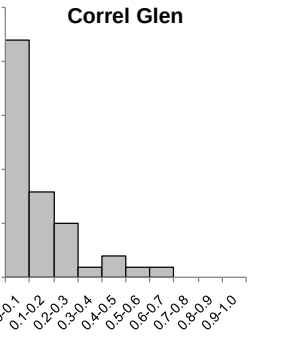

Crom
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

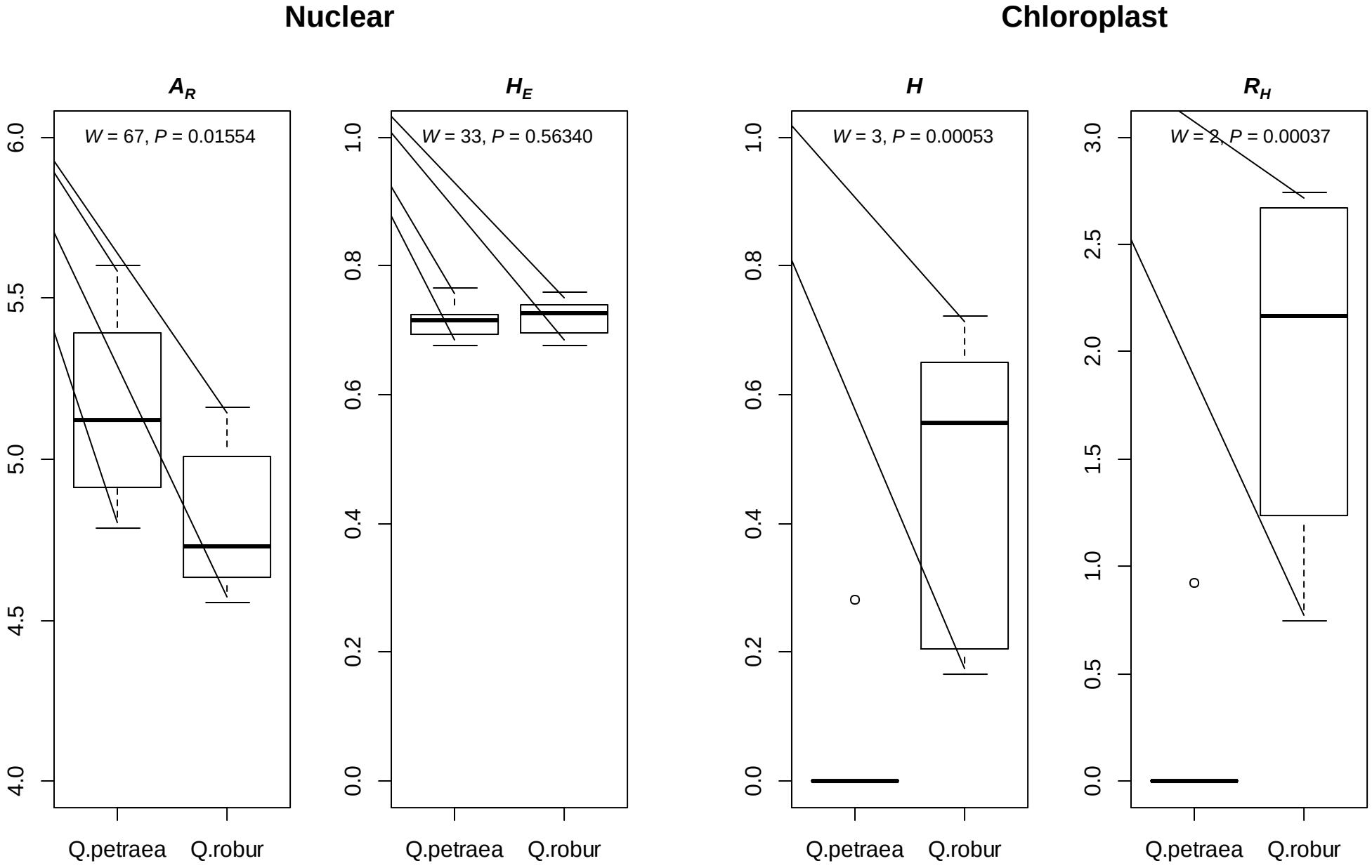

Belle Isle
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0

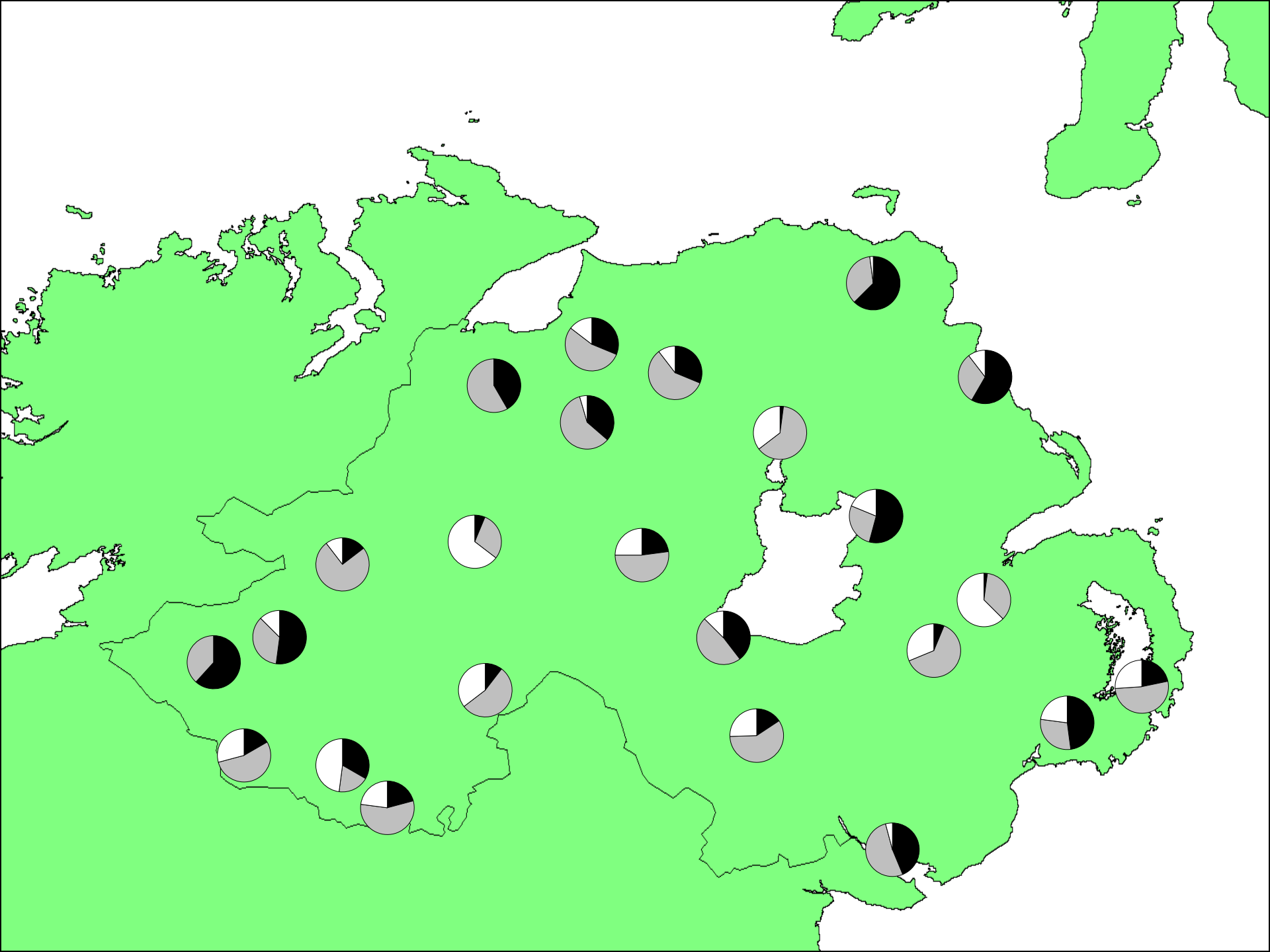

Sloughan Glen
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0


Castle Archdale
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0


Marble Arch
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0


Correl Glen
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0


Nuclear
Chloroplast
$A_R$
$H_E$
$H$
$R_H$
W = 67, P = 0.01554
W = 33, P = 0.56340
W = 3, P = 0.00053
W = 2, P = 0.00037
6.0
5.5
5.0
4.5
4.0
1.0
0.8
0.6
0.4
0.2
0.0
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Q.petraea
Q.robur



Q
0.0
0.2
0.4
0.6
0.8
1.0
Q.petraea
Q.robur
Hybrid